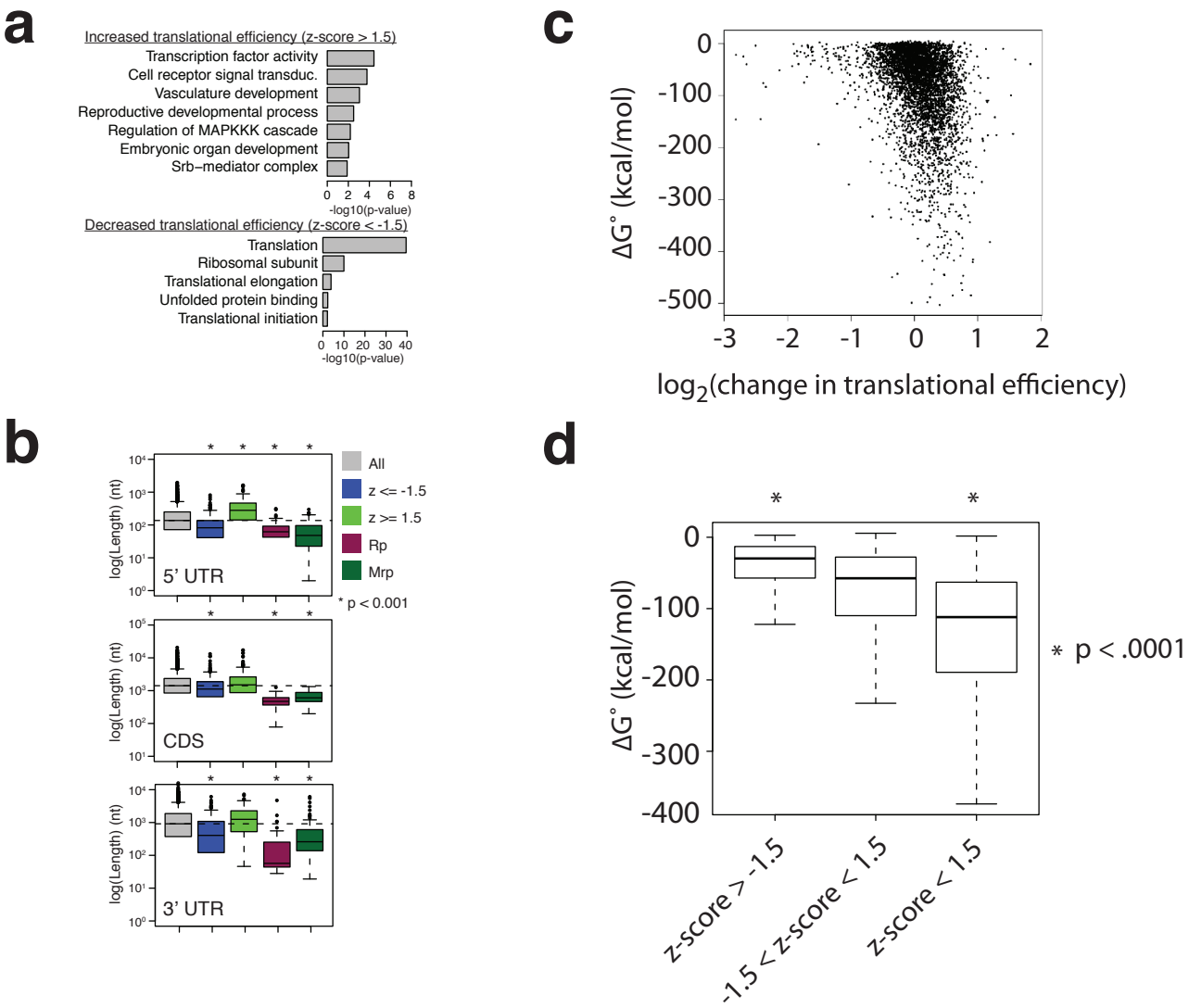
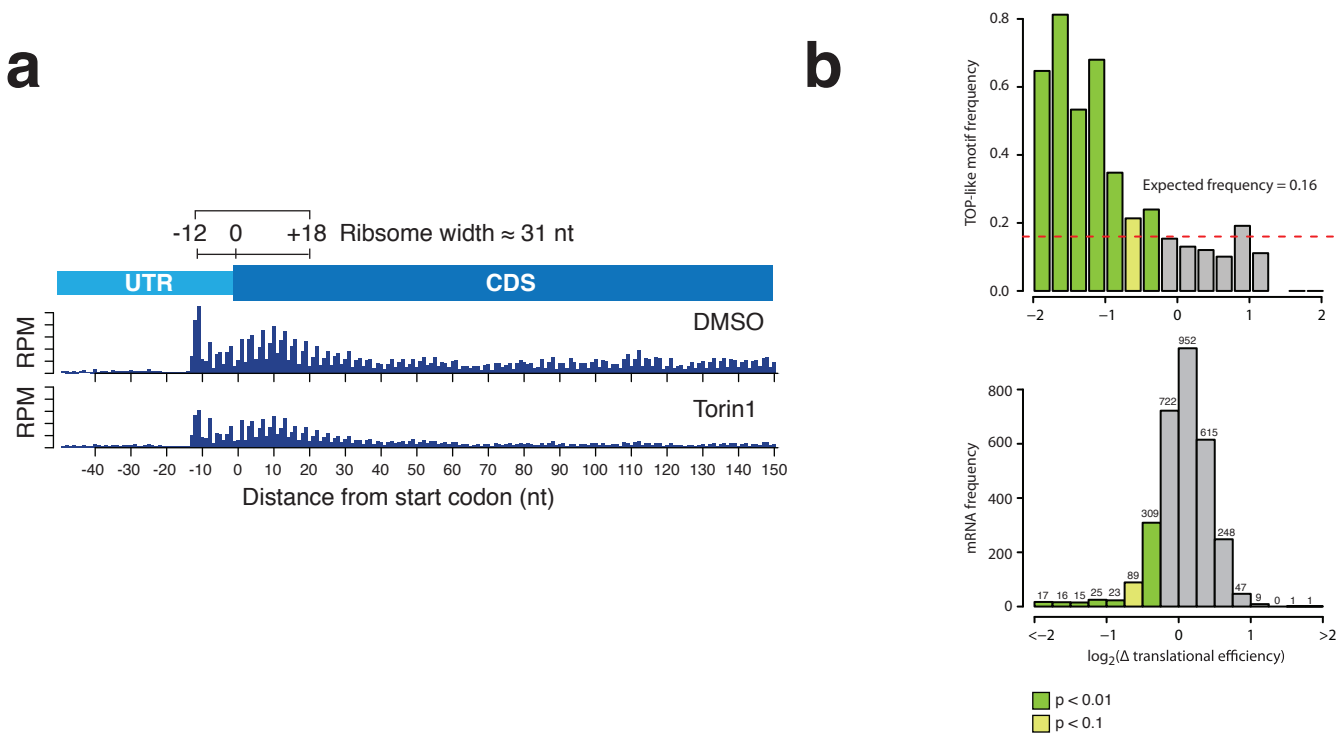


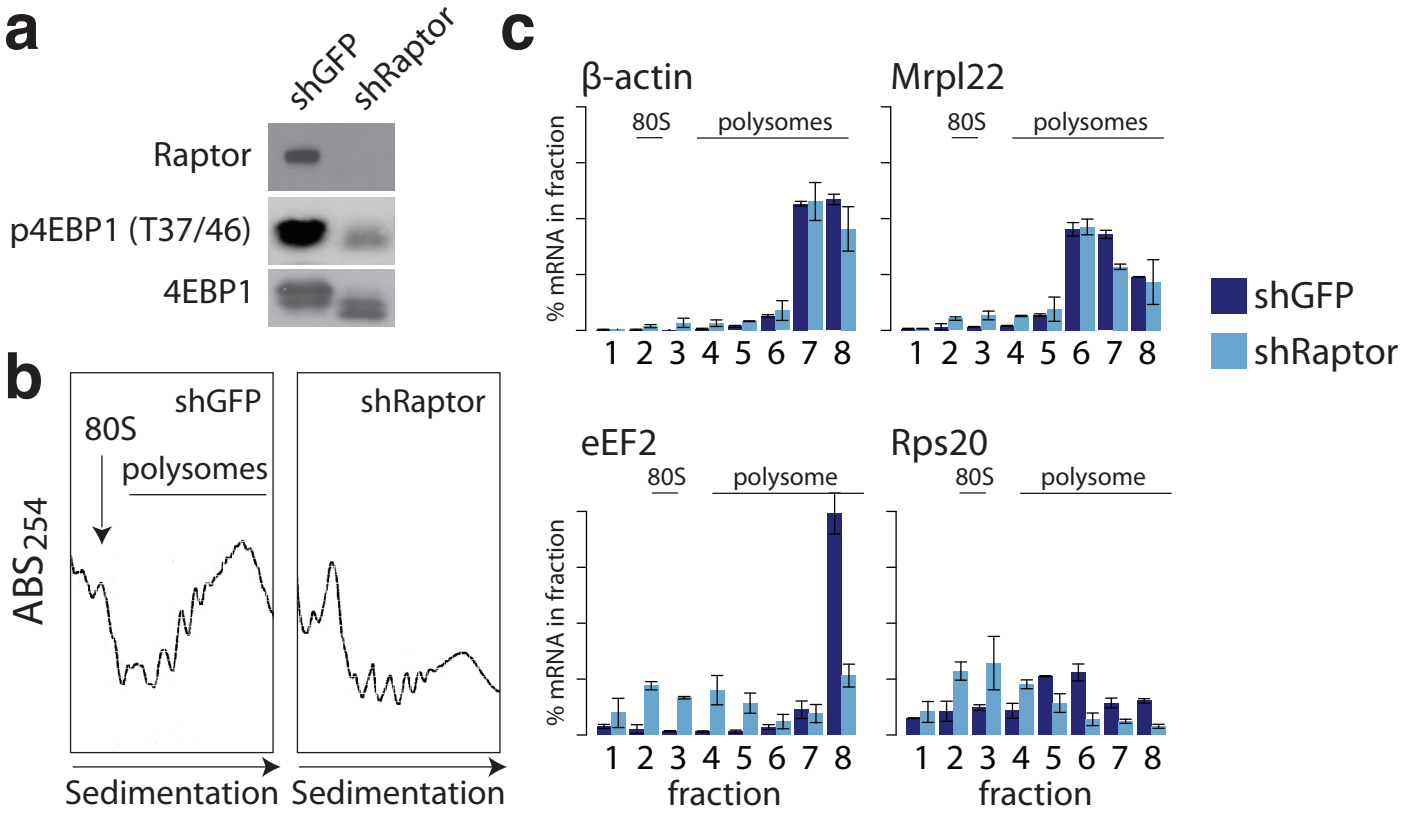
Supplementary Figure 1. Functional and sequence analysis of mTOR-regulated mRNAs

(**a**) Suppressed and resistant mRNAs from Fig. 1f were analyzed for enrichment in GO categories. (**b**) Lengths of UTRs and CDS for the following sets of mRNAs: all, Torin1-suppressed (z-score ≤ -1.5), Torin1-resistant (z-score ≥ 1.5), or encoding cytoplasmic (Rp) or mitochondrial (Mrp) ribosomal proteins. Significance was determined by the Mann-Whitney U test. (**c**) The 5' UTR folding ΔG° was plotted against the change in translational efficiency for each mRNA. (**d**) ΔG° of 5' UTRs for mRNAs with changes in translational efficiency, as indicated. Significance was determined by the Mann-Whitney U test.



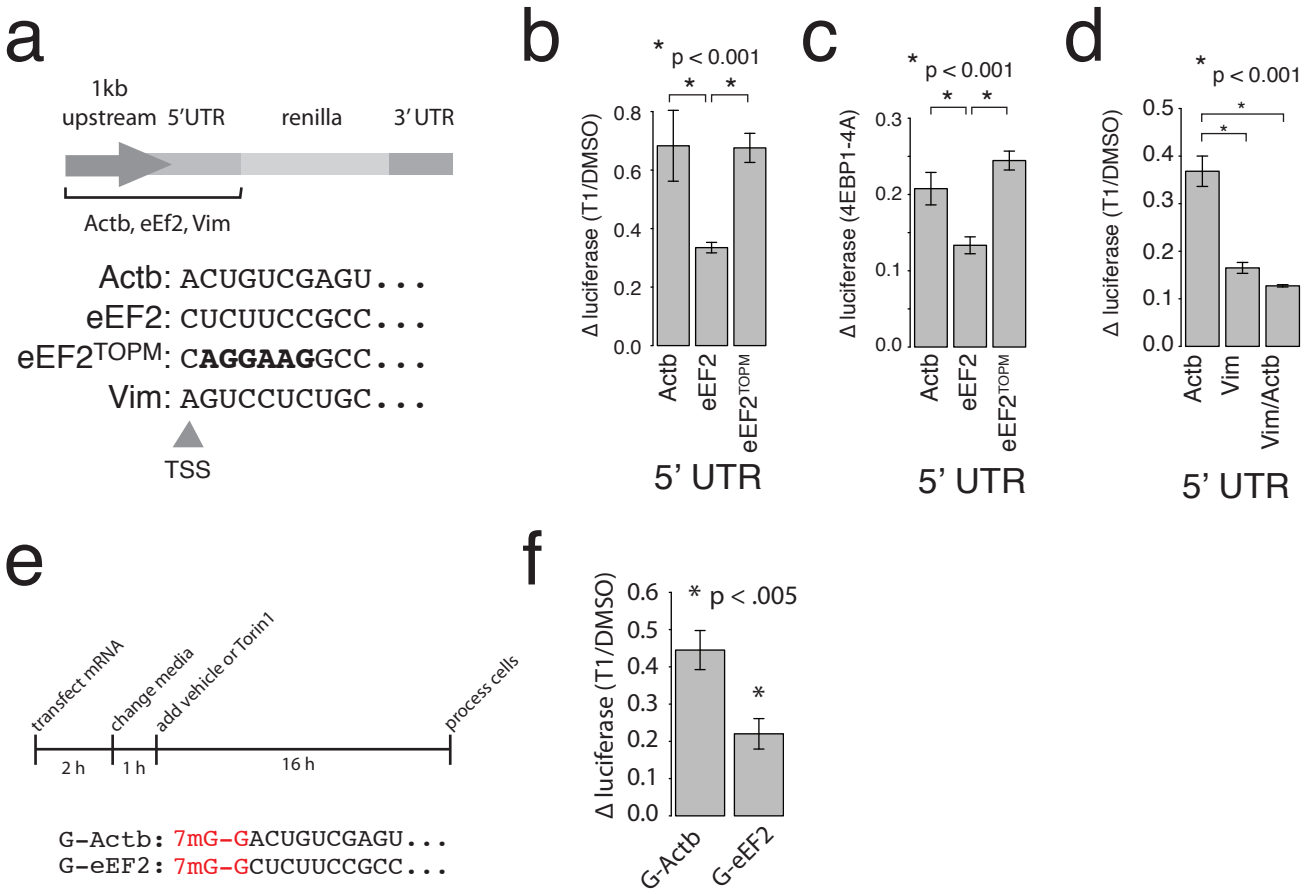
Supplementary Figure 2. Translation of mRNAs with pyrimidine-rich transcriptional start sites is suppressed by mTOR inhibition.

(**a**) Ribosome footprint reads per million (RPM) for 65 detected known TOP mRNAs in vehicle- and Torin1-treated WT MEFs. (**b**) The first 15 nt of 3089 mRNAs with confident transcriptional start site annotations in dbTSS was analyzed for the presence of a sequence of 5 pyrimidines beginning within 4 nt of the 5' terminal nucleotide. mRNAs were binned according to Torin1-dependent change in translational efficiency and the motif frequency was calculated for each bin. Significance was determined by a binomial test.



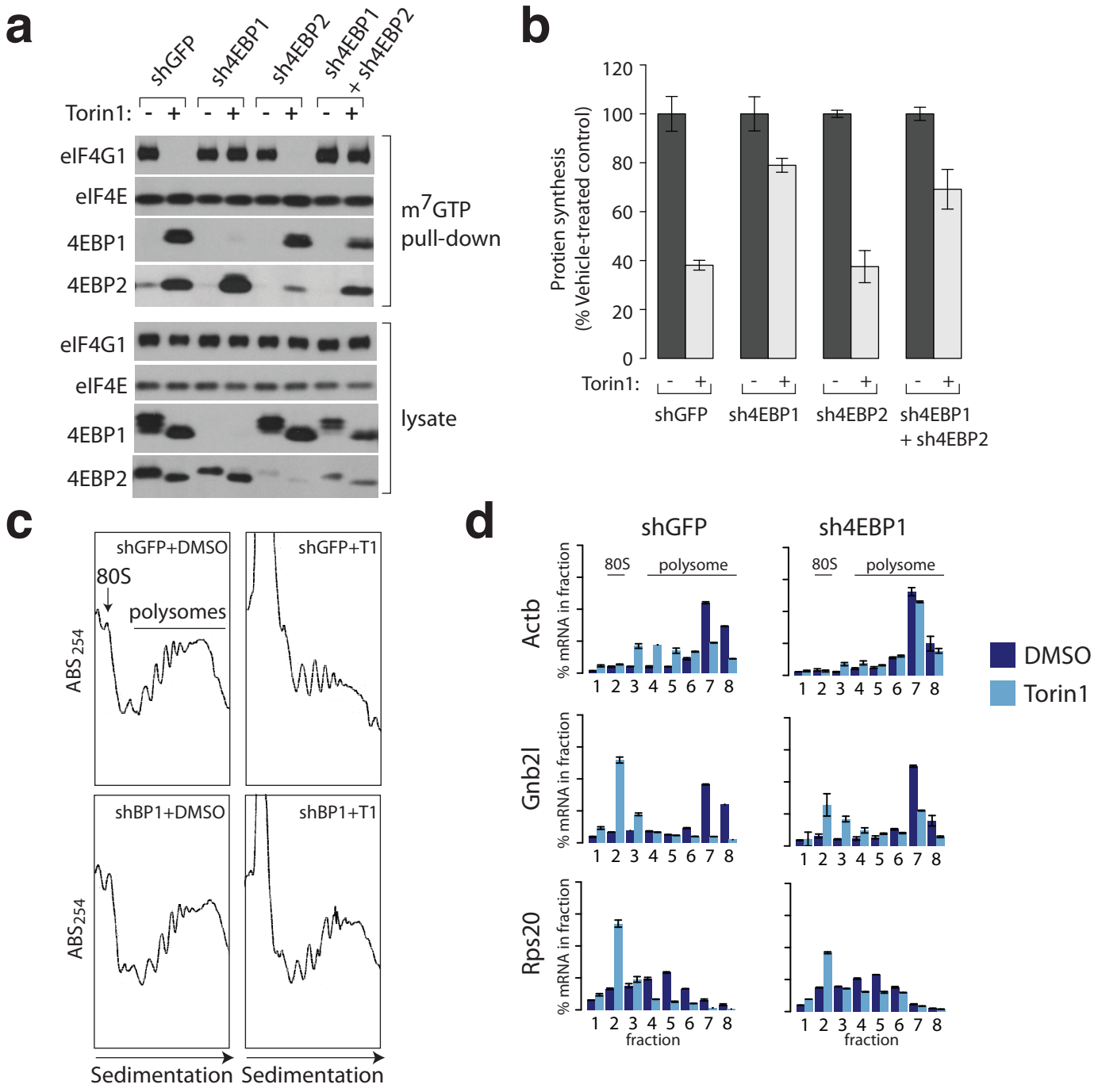
Supplementary Figure 3. Depletion of the mTORC1 component, raptor, inhibits TOP mRNA translation

(**a**) Lysates from WT MEFs expressing control (shGFP) or raptor-specific (shRaptor) shRNAs were analyzed by immunoblotting for the indicated proteins, (**b**) or by separation on 10-50% sucrose gradients. (**c**) Fractions from (b) were analyzed by QPCR for the indicated TOP (eEF2, Rps20) and non-TOP (β-actin, Mrpl22) mRNAs. Data are means +/- s.e.m.



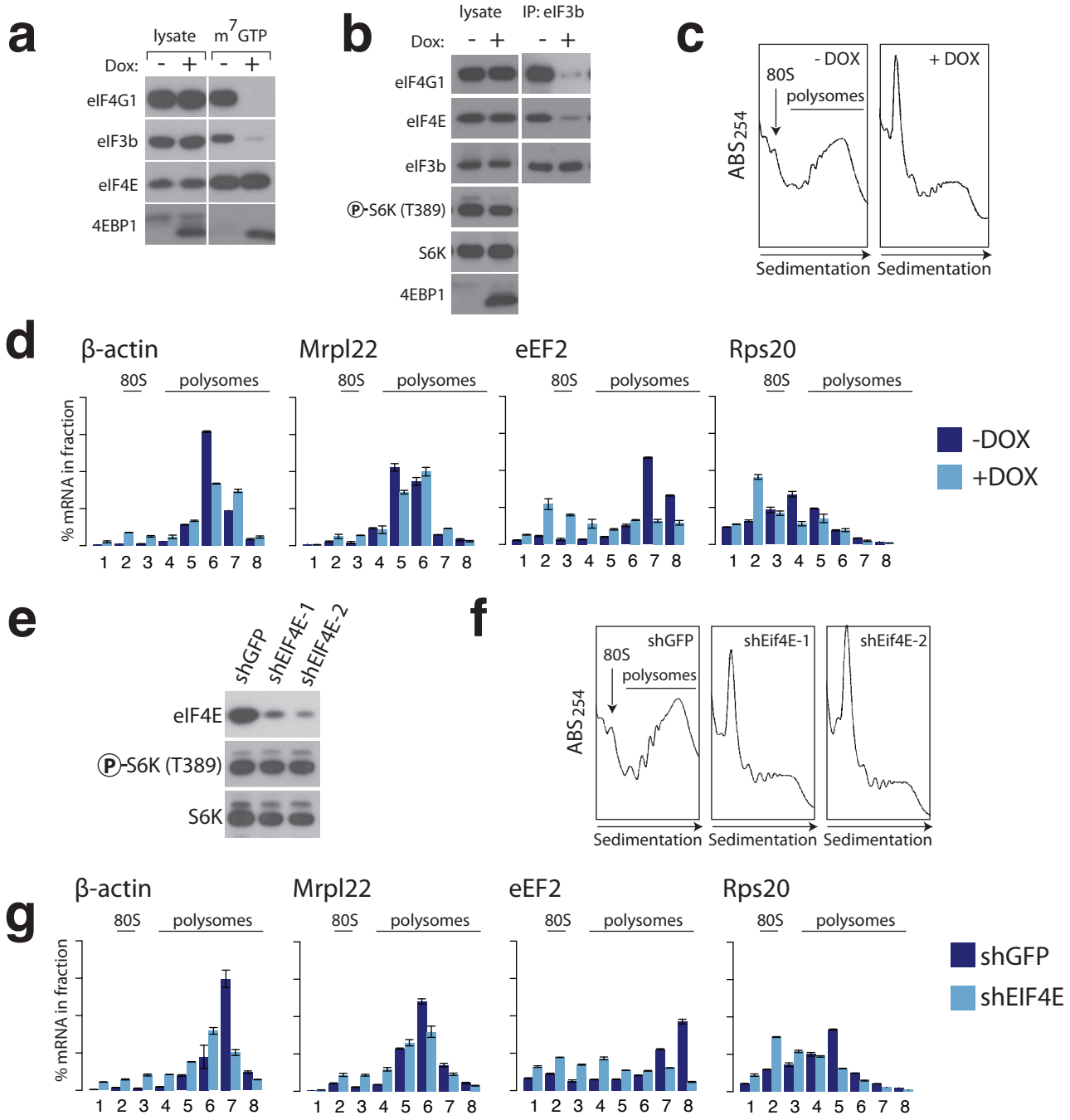
Supplementary Figure 4. TOP and TOP-like motifs are sufficient to confer mTOR-dependent translational regulation on a reporter mRNA

(**a**) Reporter constructs containing the promoter and 5' UTRs of β-actin, eEF2, eEF2 where the TOP motif has been changed to purines (eEF2^{TOPM}), and Vim followed by an ORF encoding renilla luciferase. (**b**) WT MEFs were transfected with the indicated reporter constructs for 24 h, and then treated with vehicle or 250 nM Torin1 for an additional 24 h. Cell lysates were then analyzed for luciferase activity. Data are means +/- s.e.m. Significance was determined by two-way ANOVA between indicated constructs. (**c**) WT MEFs were co-transfected with the indicated reporter constructs and either empty vector or 4EBP1-4A for 48 h. Cell lysates were then analyzed as in (b). (**d**) WT MEFs were transfected with Vim reporter from (a) or a reporter containing the promoter and first 30 nt of the Vim 5' UTR, followed by the β-actin 5' UTR (Vim/Actb), and treated as in (b). (**e**) Description of in vitro transcribed renilla luciferase mRNA containing β-actin or eEF2 5' UTRs beginning with a guanosine (G-β-actin and G-eEF2, respectively). (**f**) mRNAs from (e) were transfected into WT MEFs for 2 h. Cells were then washed, incubated for 1 h, and then treated with vehicle or 250 nM Torin1 for 16 h. Lysates were prepared and analyzed for luciferase activity. Data are means +/- s.e.m. Significance was determined by two-way ANOVA between G-β-actin and G-eEF2 5' UTR constructs.



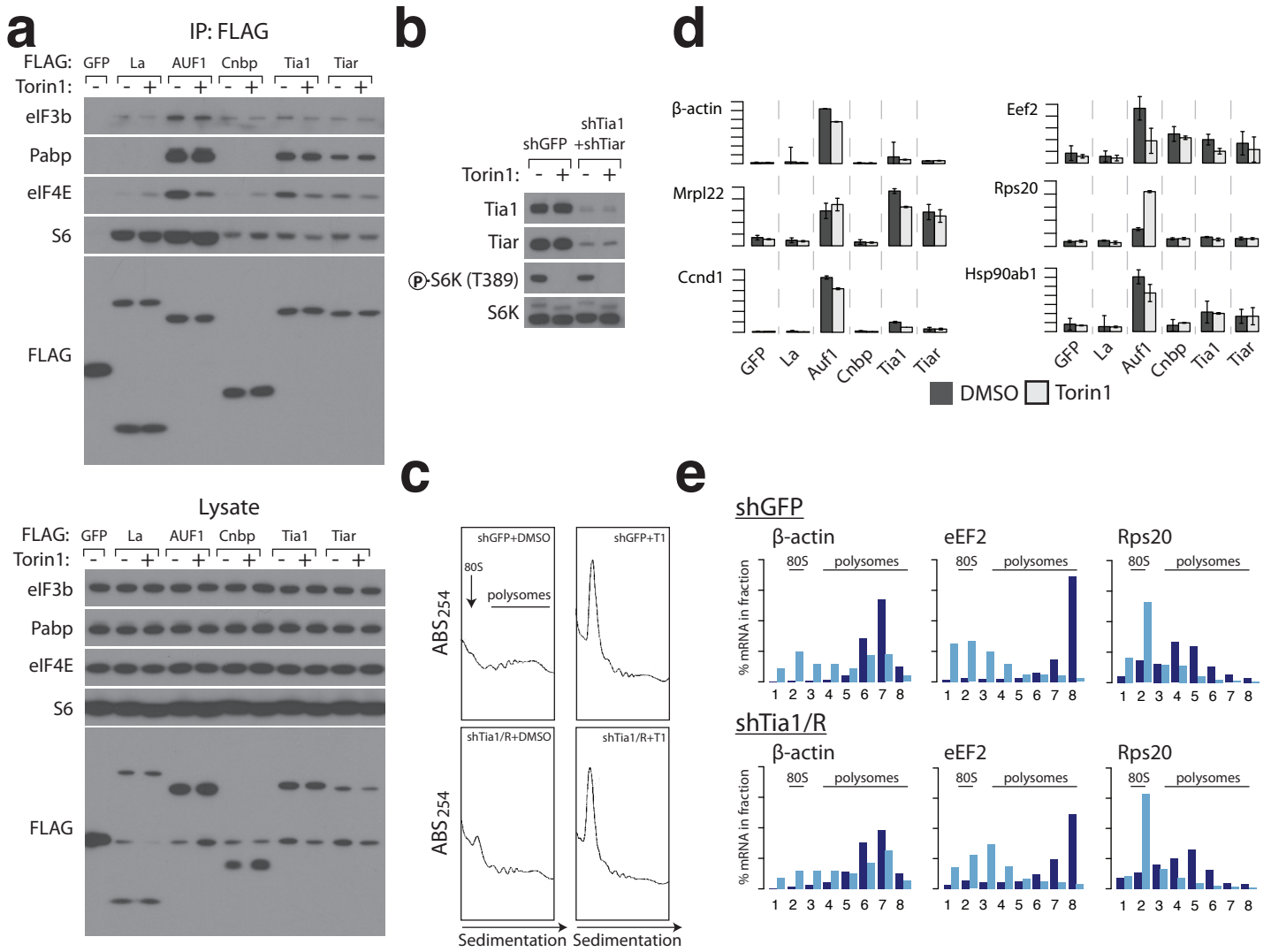
Supplementary Figure 5. 4E-BPs are required for mTOR-dependent regulation of TOP mRNAs in HeLa cells

(**a**) HeLa cells expressing control (shGFP), 4E-BP1-specific (sh4EBP1) or 4E-BP2-specific (sh4EBP2) shRNAs were treated with vehicle or 250 nM Torin1 for 2 h. Lysates were subjected to m^{7}GTP pull-downs, and analyzed by immunoblotting for the indicated proteins. (**b**) HeLa cells expressing the indicated shRNAs were treated as in (a) and then pulsed for 30 min with ^{35}S-labeled Cys/Met and lysed. ^{35}S incorporation into protein was quantified by scintillation counting and normalized to the total protein content. Data are mean +/- s.d. (n=3). (**c**) Lysates from cells expressing the indicated shRNA and treated with vehicle or 250 nM Torin1 were analyzed by separation on 10-50% sucrose gradients. (**d**) Fractions from (c) were analyzed by QPCR for the indicated TOP (Gnb2l1, Rps20) and non-TOP (β-actin) mRNAs. Data are means +/- s.e.m.



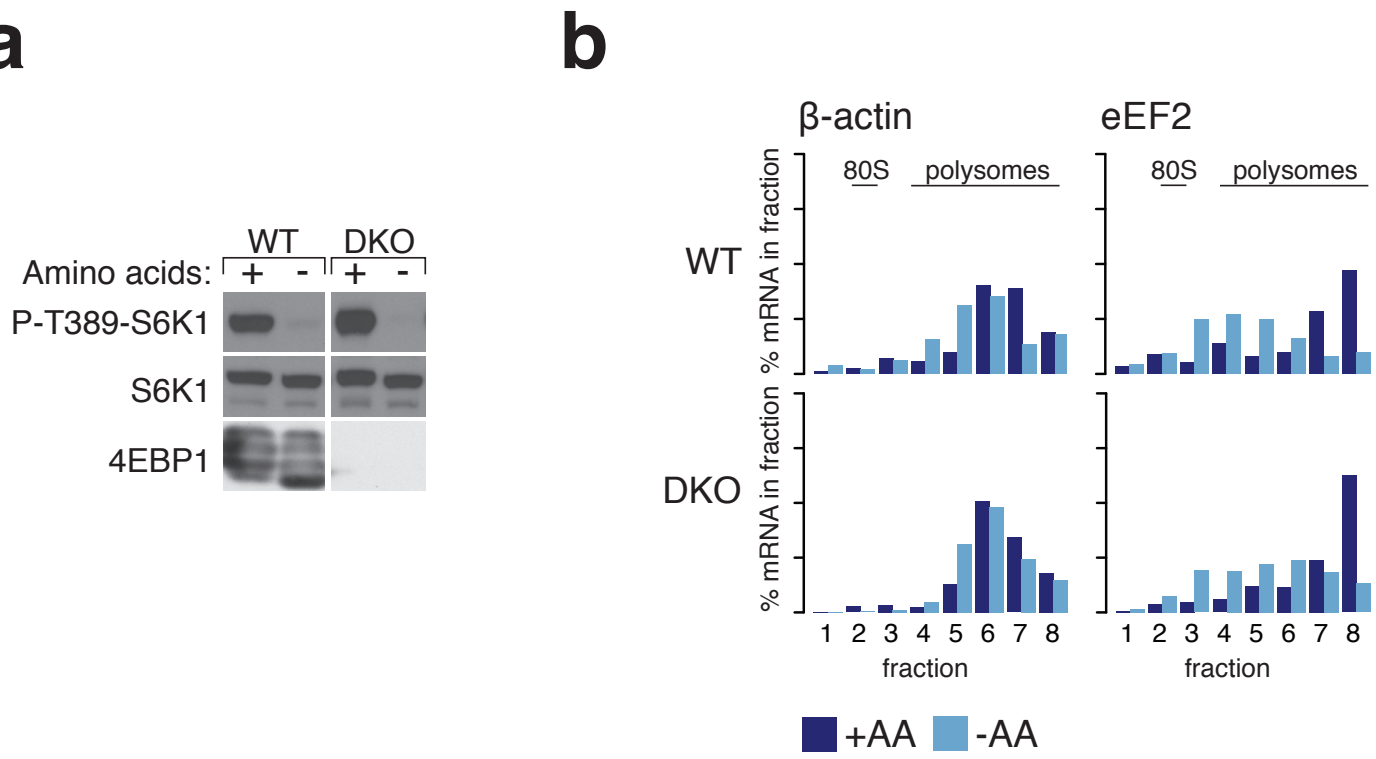
Supplementary Figure 6. TOP mRNA translation requires eIF4E and is inhibited by 4E-BPs

(**a**) WT MEFs carrying a dox-inducible 4EBP1-4A mutant construct were treated with 1 μg/ml doxycycline (Dox) for 20 h. Lysates were subjected to m^{7}GTP pull-downs, and analyzed by immunoblotting for the indicated proteins. (**b**) WT MEFs were treated as in (a) and eIF3 immunoprecipitates prepared with an antibody specific for eIF3b were analyzed by immunoblotting for the indicated proteins. (**c**) Lysates of WT MEFs treated as in (a) were analyzed by separation on 10-50% sucrose gradients. (**d**) Fractions from (c) were analyzed by QPCR for the indicated TOP (eEF2, Rps20) and non-TOP (β-actin, Mrpl22) mRNAs. Data are means +/- s.e.m. (**e**) Lysates from WT MEFs expressing control (shGFP) or eIF4E-specific (shEIF4E-1, shEIF4E-2) shRNAs were analyzed by immunoblotting for the indicated proteins, or (**f**) by separation on 10-50% sucrose gradients. (**g**) Fractions from (f) were analyzed as in (d).



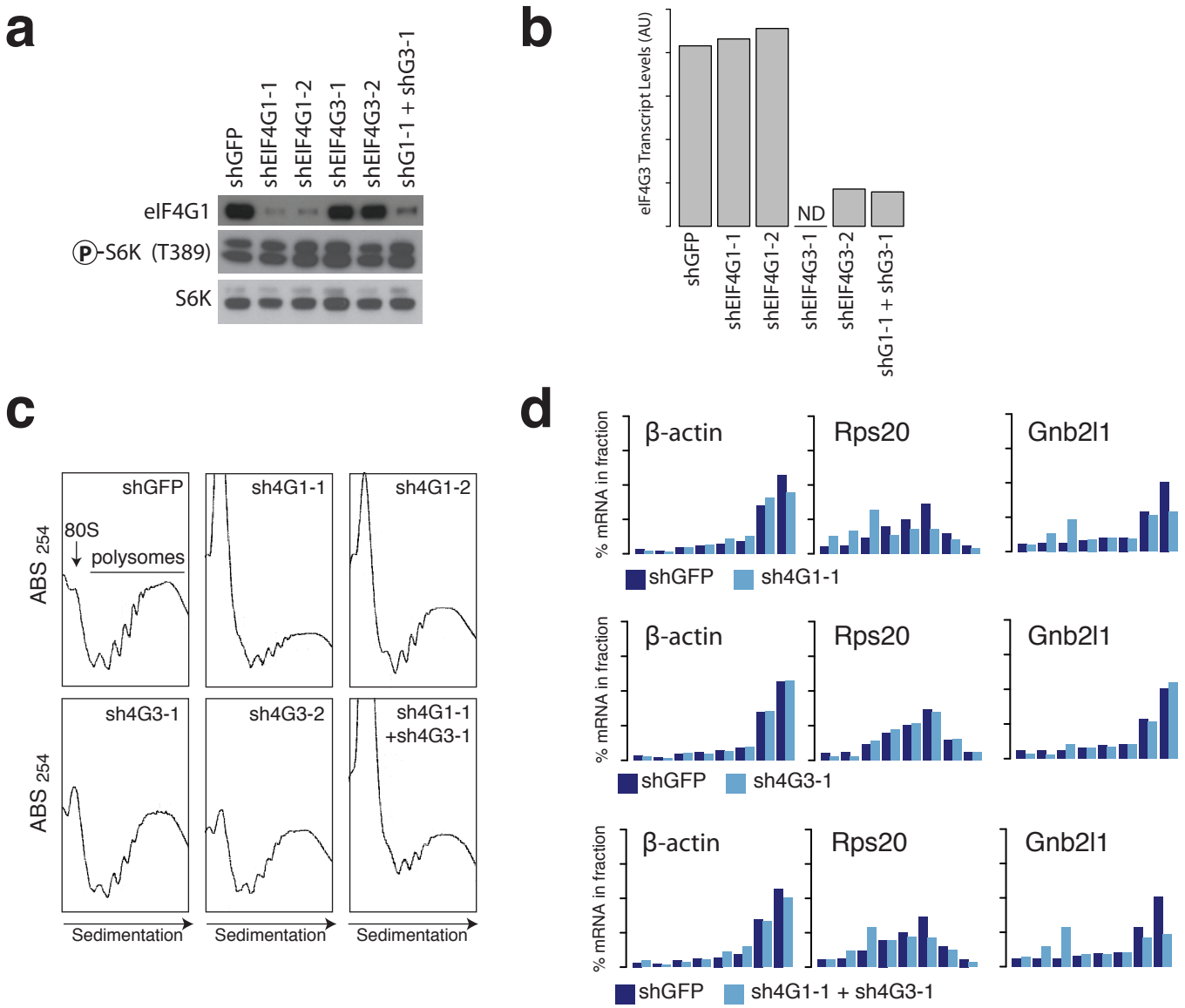
Supplementary Figure 7. Putative pyrimidine-binding proteins are not involved in mTOR-mediated regulation of TOP mRNA translation

(**a**) Lysates were prepared from WT p53$^{-/-}$ MEFs stably expressing Flag-tagged constructs of the indicated cDNAs that had been treated with vehicle or 250 nM Torin1 for 2 h. FLAG-M2 immunopurifications and lysates were analyzed by immunoblotting for the indicated proteins. (**b**) RNA was isolated from immunoprecipitations in (a) and analyzed by QPCR for the indicated TOP (eEF2, Rps20, Hsp90ab1) or non-TOP (β-actin, Mrpl22, Ccnd1) mRNAs. (**c**) Lysates were prepared from cells expressing control (shGFP) or Tia1- and Tiar-specific (shTia1+shTiar) shRNAs that were treated with vehicle or 250 nM Torin1 for 2 h, and then analyzed by immunoblotting for the indicated proteins, or (**d**) by separation on 10-50% sucrose gradients. (**e**) Fractions from (d) were analyzed for the levels of the indicated TOP (eEF2, Rps20) and non-TOP (β-actin) mRNAs.



Supplementary Figure 8. Amino acid starvation suppresses TOP mRNA translation through 4EBP-dependent and -independent mechanisms

(**a**) Wild-type (WT) and 4EBP1/2 double knockout (DKO) cells were starved for amino acids for 2 h, lysed and analyzed by immunoblotting for the indicated proteins. (**b**) Lysates from (a) were separated on 10-50% sucrose gradients and fractions were analyzed by QPCR for the indicated TOP (eEF2) and non-TOP (β-actin) mRNAs.



Supplementary Figure 9. eIF4G1 is required for the translation of TOP mRNAs in HeLa cells

(**a**) Lysates from HeLa cells expressing control (shGFP), eIF4G1-specific (shEIF4G1-1, shEIF4G1-2) or eIF4G3-specific (shEIF4G3-1, shEIF4G3-2) shRNAs were analyzed by immunoblotting for the indicated proteins. (**b**) Expression of the indicated mRNAs from cells in (a) was analyzed by QPCR. (**c**) Lysates from (a) were separated on 10-50% sucrose gradients and, (**d**) fractions were analyzed by QPCR for the indicated TOP (Gnb2l1, Rps20) and non-TOP (β-actin) mRNAs.